Distribution and metabolism of aflatoxin B_1 in the marmoset monkey (*Callithrix jacchus*)

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Whole-body autoradiography of [³H]aflatoxin B_1 (³H-AFB$_1$) in marmoset monkeys (*Callithrix jacchus*) showed a localization of bound labelling, in addition to the liver, in the nasal olfactory and respiratory mucosa and the mucosa of the nasopharyngeal duct, the pharynx, the larynx, the trachea and the oesophagus. *In vitro* microautoradiography of these tissues incubated with ³H-AFB$_1$ showed a localization of bound radioactivity in some cells in the epithelial linings of the tissues. This binding was abolished when the incubations were performed in the presence of the cytochrome P-450 inhibitor metyrapone. These results indicate a cytochrome P-450-dependent bioactivation of AFB$_1$ in some structures in the epithelia of the nasal olfactory mucosa and the upper respiratory and alimentary pathways, in addition to the liver, in the marmoset monkey. Quantitation of the *in vitro* formation of tissue-bound radioactivity from the ³H-AFB$_1$ showed that the nasal olfactory mucosa had the highest capacity to form bound metabolites among the tissues examined. Liquid chromatography of lipid-soluble metabolites formed by the nasal olfactory mucosa and the liver showed that aflatoxin M_1 (AFM$_1$) was the major metabolite. AFM$_1$ was also the major metabolite found in the liver *in vivo*. In the pigmented tissues of the marmosets there was an accumulation of non-metabolized AFB$_1$. This can be related to a melanin-affinity of AFB$_1$. The described tissues with a capacity to accumulate and metabolize AFB$_1$ may be potential targets for the carcinogenicity of this substance in the marmoset monkey.

Introduction

Aflatoxin B_1 (AFB$_1$*) is a procarcinogen requiring activation by cytochrome P-450 enzymes to form the reactive 8,9-epoxide, which binds to proteins and DNA [1,2]. It is of interest to trace tissues which have a capacity to perform the bioactivation, since these tissues may be the potential targets for the carcinogenicity of the AFB$_1$. AFB$_1$ is a potent hepatocarcinogen, but induction of tumours in other tissues has also been reported in several species [3].

We have used whole-body and microautoradiography to trace extrahepatic sites of metabolism of AFB$_1$ in mice [4,5]. The results showed that some epithelia and glands in the nose and in the upper respiratory and alimentary pathways are active in metabolizing AFB$_1$ in this species. In order to examine whether AFB$_1$ is metabolized in these tissues in a primate, the common cotton-eared marmoset (*Callithrix jacchus*) has been used in the present study to examine the tissue-disposition of AFB$_1$. This

marmoset has been increasingly used in biomedical research due to its small size and its facility to breed in captivity [6].

In the experiments G[³H]aflatoxin B_1 (³H-AFB$_1$) was given i.v. to the monkeys and the tissues accumulating and retaining unbound and tissue-bound radioactivity were then examined by whole-body autoradiography. On the basis of the results obtained, the *in vitro* formation of tissue-bound metabolites by some tissues was then examined with a microautoradiographical method. In addition, liquid chromatography (LC) and radioactivity measurements were used to identify labelled materials and to quantify the formation of unbound and tissue-bound metabolites in some tissues after *in vivo* and *in vitro* treatments with ³H-AFB$_1$.

Materials and methods

Animals

Adult female marmoset monkeys, with a body weight ~300–350 g, were used. They were fed cereals, fresh fruit and commercial pellets, and were given tapwater *ad libitum*. Three animals used for *in vivo* experiments were born in a colony at the University of Uppsala. One animal used for *in vitro* experiments was obtained from Hässle Läkemedel AB (Mölndal, Sweden).

Chemicals

³H-AFB$_1$ with a specific activity of 30 Ci/mmol was obtained from Moravek Biochemicals (Brea, CA, USA). The ³H-AFB$_1$ was purified and cleaned up to a purity of >98% by LC using a system described previously [7]. AFB$_1$, aflatoxin B_{2a} (AFB$_{2a}$), -M_1 (AFM$_1$), -P_1 (AFP$_1$), -Q_1 (AFQ$_1$) and aflatoxicol (AFL) were purchased from SIGMA Chemical Co. (St Louis, MO, USA). Metyrapone was a gift from Ciba-Geigy (Basel, Switzerland). Other chemicals used in the study were of analytical grade and obtained from regular commercial sources.

In vivo experiments

Three monkeys used for whole-body autoradiography were anesthetized by i.m. injection of ketamine chloride (10 mg/kg body wt) prior to i.v. injections (*vena saphena*) with ³H-AFB$_1$, dissolved in 50 μl ethanol. Each animal received a dose of 2 μg AFB$_1$/kg body wt. The animals were killed by overdoses of ketamine chloride 15 min, 2 and 6 h after the ³H-AFB$_1$ injections. They were then embedded in aqueous carboxymethyl cellulose and sectioned for whole-body autoradiography as described by Ullberg [8].

To study the distribution of firmly bound radioactivity, every other freeze-dried tissue section was extracted successively with 5% trichloroacetic acid, 50% ethanol, 99.5 % ethanol and heptane for 1 min, and then rinsed with tap-water for 5 min. The sections were dried and pressed against X-ray films (Ultrofilm; LKB, Bromma, Sweden) together with the adjacent non-extracted sections.

Samples of the liver and the eye-melanin were taken from the remaining carcasses (which had been kept at −20° C) of the animals used for the whole-body sectioning. The tissues were homogenized in 50 mM phosphate buffer:methanol (1:1) and extracted with chloroform and buffer until no more radioactivity could be removed from the tissues, as described earlier [7]. For each tissue all chloroform and buffer extracts were pooled separately, and the radioactivity-contents were measured by liquid scintillation counting. The tissue-pellets remaining after extractions were dissolved in 1M NaOH. The amounts of tissue-bound radioactivity were quantified by liquid scintillation counting, and the protein contents were determined according to Lowry *et al.* [9].

The presence of unmetabolized AFB$_1$ and AFB$_1$-metabolites in the chloroform extracts was analyzed by LC on a 30 cm long column packed with LiChrosorb RP-18, diameter 5 μm, as described earlier [7]. Two systems were used: In system A, the eluent was 30% acetonitril buffered to pH 3.0. In this system AFB$_1$, AFL and AFB$_{2a}$ eluted as well separated peaks, whereas AFM$_1$, AFP$_1$ and AFQ$_1$ were eluted together. In system B 43% methanol buffered to pH 3.0 was used as eluent. AFM$_1$, AFP$_1$ and AFQ$_1$ separated well in this system.

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In vitro experiments

Pieces of the nasal olfactory mucosa of an ethmoturbinate, nasal respiratory mucosa of the nasal septum, nasopharyngeal duct, trachea, pharynx, oesophagus and liver of an untreated monkey were incubated with 2 μCi (77 pmol) ^{3}H-AFB$_1$ in 2.5 ml 50 mM phosphate buffer, pH 7.4, containing 3 mM MgCl$_2$, 60 mM KCl, 0.4 mM NADP and 3 mM glucose-6-phosphate. The incubations were carried out for 1 h at 37° C under O$_2$ or in O$_2$-atmosphere with the cytochrome P-450 inhibitor metyrapone (0.5 mM) added to the medium. After the incubation the pieces were fixed in 4% formaldehyde in phosphate buffer pH 7.4. The tissues were dehydrated in ethanol series and embedded in Technovit 7100 (Heraeus Kulzer GmbH, Wehrheim, Germany). Two μm thick sections were cut on glass slides and covered with Kodak NTB-2 emulsion by dipping. The sections were stained with hematoxylin-eosin or according to the periodic acid-Schiff technique as described earlier (4). The extensive extractions during the fixation and embedding procedures will remove all unbound radioactivity (5), and the autoradiograms will, therefore, show only tissue-bound radioactivity.

In an additional experiment slices of the nasal olfactory mucosa, nasal respiratory mucosa, mucosa of the nasopharyngeal duct and pharynx, and tracheal and oesophageal mucosa were incubated with 0.2 μCi (7.7 pmol) ^{3}H-AFB$_1$ under the same conditions as for the *in vitro* microautoradiography. The incubations were stopped after 1 h by adding 2.5 ml methanol. The tissue slices were homogenized and extracted with chloroform and buffer in the same manner as described above for the tissue pieces taken from the i.v. injected animals. The tissue pellets remaining after the extractions were dissolved in 1 M NaOH. The amount of radioactivity was analyzed by liquid scintillation counting, and the protein contents were measured according to Lowry *et al.* (9). The presence of AFB$_1$ and AFB$_1$-metabolites in the chloroform extracts of the liver and the nasal olfactory mucosa was determined by LC as described above.

Results

In vivo experiments

The whole-body autoradiograms of the freeze-dried sections of the monkey killed 15 min after i.v. injection of ^{3}H-AFB$_1$ showed a considerable labelling of the blood (Figure 1A). A homogeneous distribution of radioactivity at a level about equal to that in the blood was present in most tissues of the body, whereas radioactivity markedly exceeding this labelling was seen in a few tissues. This applied to the liver parenchyma, the melanin of the eyes, and the nasal olfactory mucosa (Figures 1A and 2A). There was also a strong radioactivity in the contents of the gall bladder and bile ducts, in the pelvis of the kidneys and in the urinary bladder. The nasal respiratory mucosa, the mucosa of the nasopharyngeal duct, the pharynx, the larynx, the trachea and the oesophagus, and the melanin of hair-follicles showed a labelling which somewhat exceeded the level in the blood, and this also applied to the cortex of the kidney. In the central nervous system there was a low labelling of the white matter, whereas in the grey matter the radioactivity somewhat exceeded the level in the blood (Figures 1A and 2A).

Whole-body autoradiograms of sections extracted in trichloroacetic acid, organic solvents and water of the monkey killed 15 min after i.v. injection of ^{3}H-AFB$_1$ showed retention of considerable radioactivity only in a few tissues. This applied to the nasal olfactory mucosa and the liver parenchyma—the level being slightly higher in the former than in the latter tissue—and to the pigmented parts of the eyes (Figure 1B and 2B). Retained radioactivity was also present in the melanin of hair-follicles and, at a considerably lower level than the previously mentioned sites, in the nasal respiratory mucosa and the mucosa of the nasopharyngeal duct, the pharynx, the larynx, the trachea and the oesophagus. A low level of bound radioactivity was also present in the blood.

In whole-body autoradiograms of freeze-dried sections of the monkey killed 2 h after i.v. injection of ^{3}H-AFB$_1$ the homogeneous labelling of most tissues, which was seen at the 15 min interval, had disappeared. Radioactivity was still detectable in the blood (Figures 3A and 4A). Labelling exceeding this radioactivity was seen in the liver parenchyma, in the pigment

of the eyes and the hair-follicles, in the nasal olfactory and respiratory mucosae, in the mucosa of the nasopharyngeal duct, the pharynx, the larynx, the trachea and the oesophagus (Figures 3A and 4A). There was also a marked labelling of the kidney and the contents of the gall-bladder and the intestines.

In whole-body autoradiograms of extracted sections of the monkey killed 2 h after i.v. injection of ^{3}H-AFB$_1$ radioactivity was retained in the liver, in the pigmented tissues, in the nasal olfactory mucosa and in the described mucosal linings of the upper respiratory and alimentary tracts (Figure 3B).

In whole-body autoradiograms of freeze-dried sections of the monkey killed 6 h after i.v. injection of ^{3}H-AFB$_1$ similar distribution-pictures were seen as those described for the autoradiograms of freeze-dried sections of the animal killed after
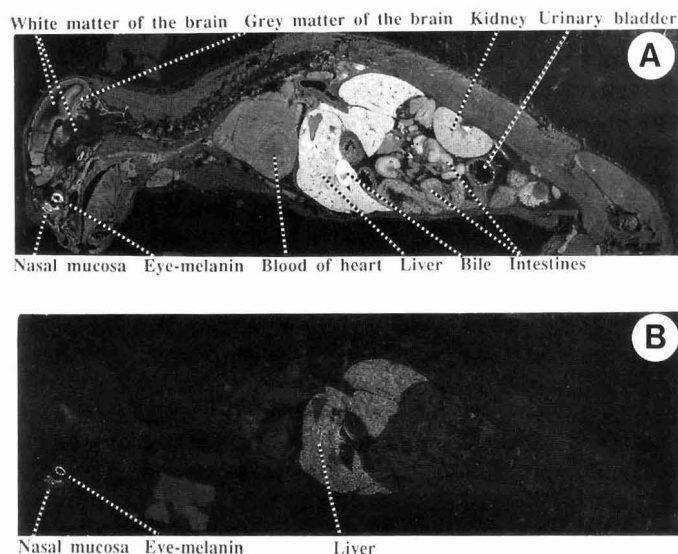
White matter of the brain Grey matter of the brain Kidney Urinary bladder

Nasal mucosa Eye-melanin Blood of heart Liver Bile Intestines

Nasal mucosa Eye-melanin Liver

Fig. 1. Whole-body autoradiograms of a freeze-dried section (**A**) and an adjacent extracted section (**B**) of a marmoset monkey killed 15 min, after an i.v. injection of ^{3}H-AFB$_1$ (2 μg/kg body wt).

Nasal mucosa Grey matter of the brain Eye-melanin Nasopharyngeal duct

Eye-melanin White matter of the brain Eye-melanin Nasal mucosa Blood

Nasal mucosa Eye melanin Nasal mucosa Nasopharyngeal duct
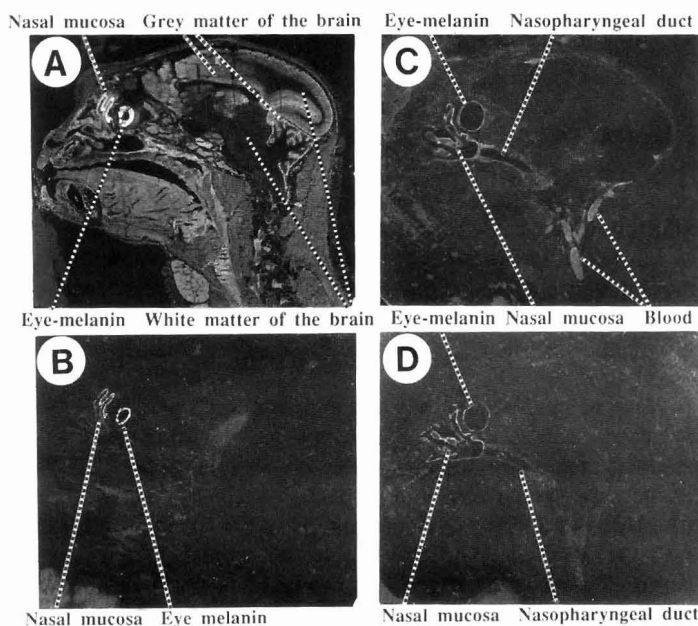
Fig. 2. Details of whole-body autoradiograms of freeze-dried sections (**A,C**) and adjacent extracted sections (**B,D**) of marmoset monkeys killed 15 min (**A,B**) and 6 h (**C,D**) after i.v. injections of ^{3}H-AFB$_1$ (2 μg/kg body wt).

2 h (Figures 2C and 4B). Autoradiograms of extracted sections of the animal killed after 6 h also showed distribution-pictures similar to those seen with extracted sections of the monkey killed after 2 h (Figure 2D).

The extractions and LC-analyses of liver pieces obtained from the monkeys used for the whole-body autoradiography showed that at the 15 min survival interval most of the radioactivity represented unmetabolized AFB_1 (Table I). At 2 h, the major part of the radioactivity was found to be water-soluble metabolites, whereas at 6 h tissue-bound metabolites constituted the highest proportion of the radioactivity in the liver. It can further be seen from Table I that the chloroform-soluble metabolites show the highest levels at 15 min and the values then decrease at 2 and 6 h.

LC analysis of the chloroform-extractable radioactivity in the liver at 15 min (Figure 5) and 2 h (data not shown) showed that AFM_1 was the dominating metabolite. Small amounts of AFP_1 were also detected.

LC analysis of the chloroform-extractable radioactivity in the eye-melanin showed that most of the radioactivity was unmetabolized AFB_1 (Figure 6). Thus, 98, 96 and 90% of the

total radioactivity coeluted with AFB_1-reference of 15 min, 2 and 6 h respectively. Small amounts of AFP_1, AFM_1 and AFQ_1 were also observed, with increased levels at increasing survival intervals.

In vitro experiments

In the microautoradiograms of the nasal olfactory mucosa, obtained from the AFB_1-incubations without metyrapone, a strong labelling was present in the cells of Bowman's glands in the lamina propria mucosae (Figure 7A). There was also a marked labelling of sustentacular and neuronal cells in the surface epithelium. Both in Bowman's glands and in the surface epithelium there was a preferential labelling of the nuclei of the cells. Figure 6B shows that there is no specific labelling in incubations in the presence of metyrapone. Incubations without metyrapone with tissues of the nasal respiratory mucosa, the mucosa of the nasopharyngeal duct and pharynx, and the tracheal and oesophageal mucosa showed a labelling of cells in the surface epithelium. Incubations of these tissues in the presence of metyrapone showed lack of specific labelling (data not shown). LC analysis of chloroform-extracts of the nasal olfactory mucosa and the liver incubated with ^3H-AFB_1 showed that AFM_1 was the major lipid-soluble metabolite (data not shown).

The level of tissue-bound metabolites formed during the in vitro incubations was highest in the nasal olfactory mucosa (Table II). Thereafter the liver, the mucosa of the nasopharyngeal duct, the nasal respiratory mucosa and the oesophageal mucosa followed in decreasing order.

Discussion

The results of the present study indicate that some structures in the epithelia of the nasal olfactory mucosa and the upper respiratory and alimentary pathways, in addition to the liver, have a capacity to bioactivate AFB_1 in the marmoset monkey. Our results showed that the accumulation of bound metabolites in the tissues in vivo correlated with a capacity of the same tissues to form tissue-bound AFB_1-metabolites in vitro. The observations that the in vitro-binding of metabolites was inhibited by the cytochrome P-450 inhibitor metyrapone indicates a cytochrome P-450-dependent bioactivation of AFB_1 in these tissues.

Disposition and metabolism of AFB_1 in the nasal olfactory mucosa and in the upper respiratory and alimentary pathways have been shown previously in some other species. Thus, we have previously found a much higher metabolism of AFB_1 in the nasal olfactory mucosa than in the liver of cattle and mice (5,7,11). In vitro metabolism of AFB_1 has been shown in tracheal explants of rhesus monkeys (Macaca mulatta), rabbits, hamsters and rats (10). Autoradiographic studies with AFB_1 in mice (5) and rats (P.Larsson and H.Tjälve, unpublished observations) have shown localization of bound material in the tracheal epithelium in vivo in these species. The results of our study in the mouse (5) indicated a capacity also of the nasal respiratory mucosa and the mucosa of the nasopharyngeal duct and the
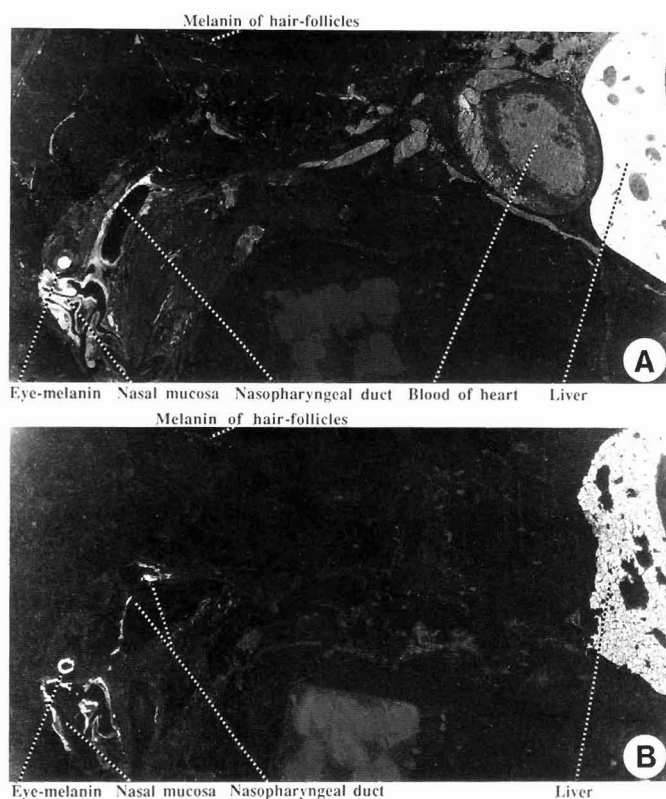

Fig. 3. Whole-body autoradiograms of a freeze-dried section (**A**) and an adjacent extracted section (**B**) of a marmoset monkey killed 2 h after an i.v. injection of ^3H-AFB_1 (2 µg/kg body wt).

Table I. Levels of AFB_1 and AFB_1-metabolites in the liver of marmoset monkeys killed 15 min, 2 and 6 h after i.v. injection of ^3H-AFB_1. Values are expressed as mean ± SD of 4 samples from each animal. Figures within brackets are the percentages of the total radioactivity at each survival time.

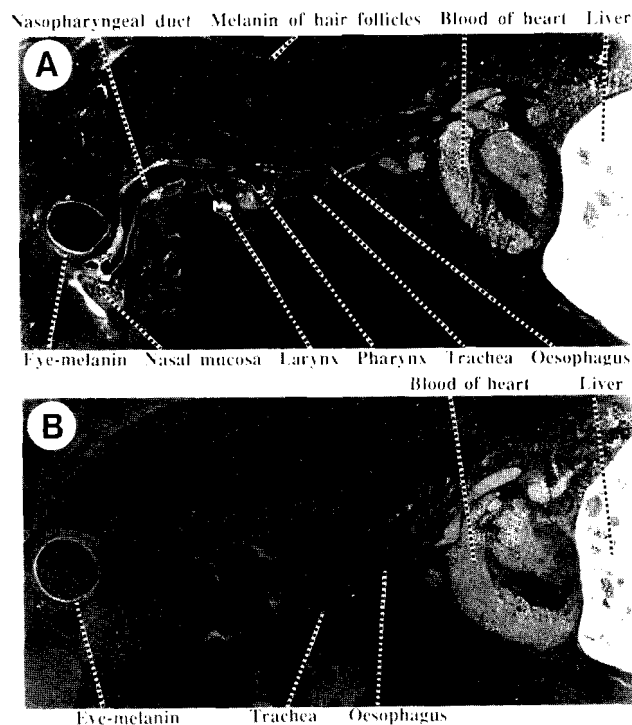| Survival time	Levels of AFB_1 and AFB_1-metabolites (pmol·10^3/mg protein)			
	AFB_1	chloroform-soluble	water-soluble	tissue-bound
15 min	122 ± 3 (49)	26 ± 5 (10)	77 ± 8 (30)	27 ± 3 (11)
2 h	3 ± 1 (2)	9 ± 3 (5)	92 ± 2 (54)	66 ± 3 (39)
6 h	2 ± 1 (1)	5 ± 3 (6)	38 ± 2 (43)	44 ± 2 (50)

Fig. 4. Whole-body autoradiograms of freeze-dried sections of marmoset monkeys killed (**A**) 2 h and (**B**) 6 h after i.v. injections of ^{3}H-AFB$_1$ (2 μg/kg body wt).
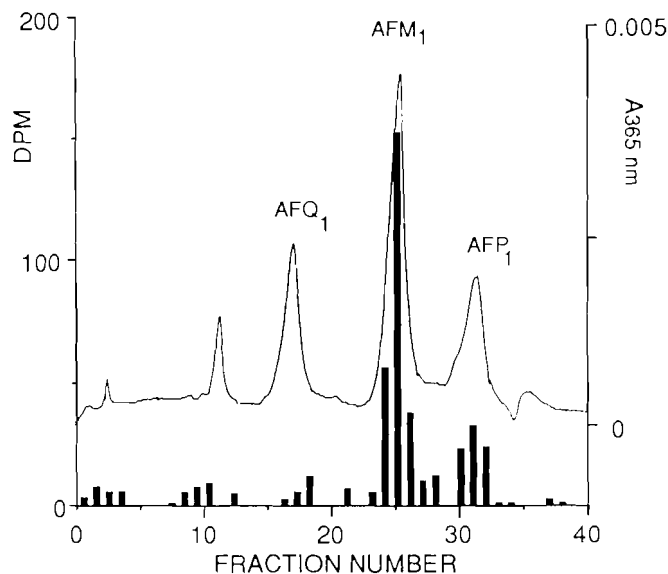

Fig. 5. LC-separation of chloroform-extractable metabolites in the liver at 15 min. after i.v. injection of ^{3}H-AFB$_1$. The LC-system B was applied to separate the metabolites AFM$_1$, AFP$_1$ and AFQ$_1$. For further details, see Materials and methods. Absorbance at 365 nm (——) and amount of ^{3}H (I) in the fractions are shown.

Fig. 6. LC-separation of chloroform-extractable radioactivity in the eye-melanin at 2 h after i.v. injection of ^{3}H-AFB$_1$. The LC-system A was used; for further details, see Materials and methods. Absorbance at 365 nm (——) and amount of ^{3}H (I) in the fractions are shown.

Fig. 7. Microautoradiograms of the nasal olfactory mucosa from a marmoset monkey. The nasal tissues were incubated for 1 h in a buffer containing ^{3}H-AFB$_1$ (0.003 μM) in (**A**) O$_2$-atmosphere or (**B**) O$_2$-atmosphere with metyrapone (0.5 mM) present in the incubation medium. (s = sustentacular cells; n = neuronal cells; Bg = cells of Bowman's gland.) (PAS-hematoxylin; magnification ×500.)

oesophagus to metabolize AFB$_1$. Cytochrome P-450 enzymes have been shown in the nasal olfactory mucosa and in the epithelial linings of the upper respiratory and alimentary pathways in several species (12 – 15). These tissues are also prevalent sites for the metabolism of some chemical carcinogens, such as N-nitrosamines (16). Localization of bound metabolites from the tobacco-specific N-nitrosamines N-nitrosonornicotine and
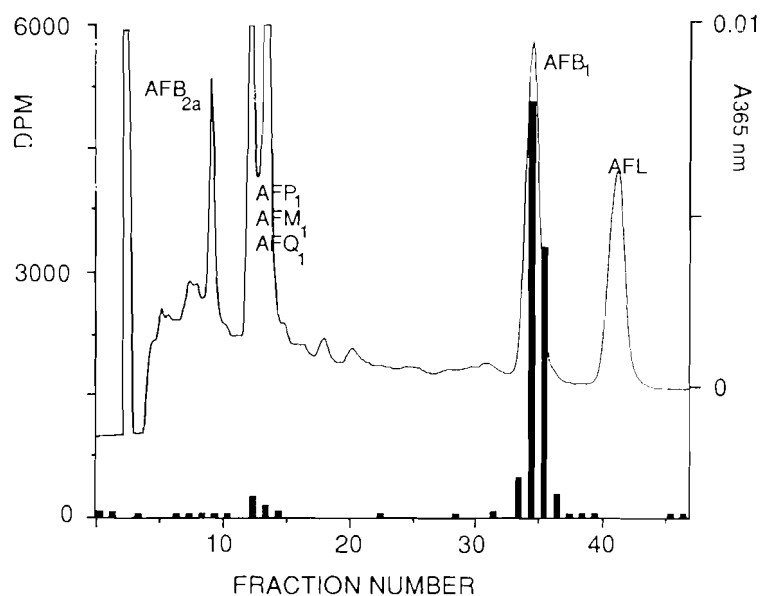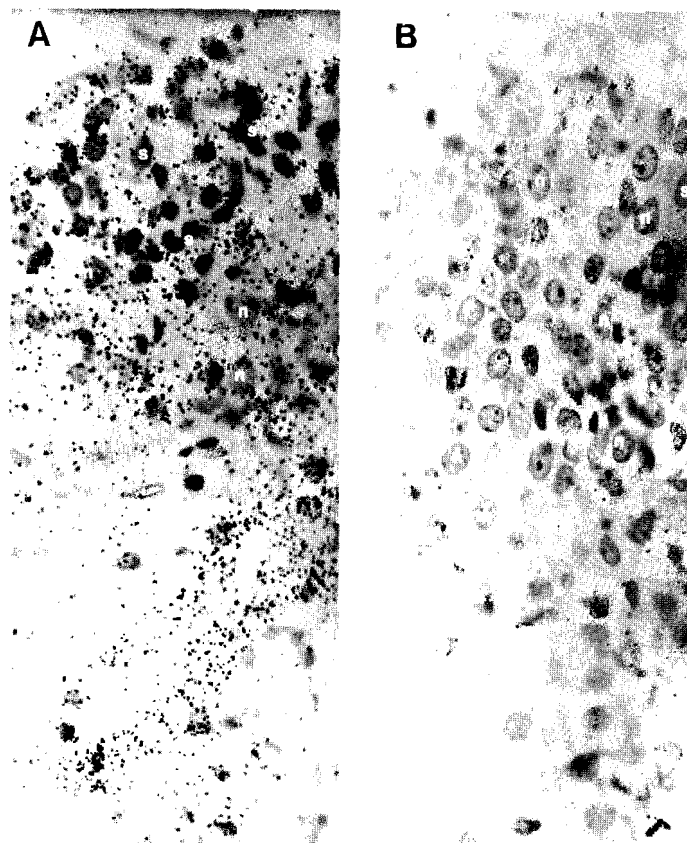
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Table II. Formation of tissue-bound AFB$_1$-metabolites from ^{3}H-AFB$_1$ *in vitro*.

	pmol tissue-bound AFB$_1$-metabolites/mg protein
Nasal olfactory mucosa	1.10
Liver	0.82
Mucosa of the nasopharyngeal duct	0.55
Nasal respiratory mucosa	0.35
Oesophageal mucosa	0.21
Control	0.08

Tissue-slices of various tissues of a marmoset monkey were incubated at $+37°C$ with 0.2 μCi ^{3}H-AFB$_1$ (7.7 pmol) for 1 h and the amounts of bound metabolites formed were then determined. The values are from single incubations, except for the control (boiled liver; $n = 2$).

4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, which are activated by cytochrome P-450-enzymes, has been shown in the nasal mucosa in the marmoset (17).

The extrahepatic tissues are heterogeneous and high cytochrome P-450 activities may be confined to specific cell-types. The microautoradiography of ^{3}H-AFB$_1$ in the nasal olfactory mucosa of the marmoset showed a strong labelling of Bowman's glands in the lamina propria mucosae and a somewhat weaker labelling of sustentacular and neuronal cells in the surface epithelium. Our previous studies with ^{3}H-AFB$_1$ have shown a labelling of the cells of Bowman's glands *in vitro* in cattle (7) and *in vivo* in young mice (5). In the cattle, there was also a labelling of the sustentacular cells (7). In mice the sustentacular cells showed a stronger labelling at *in vitro* incubations with ^{3}H-AFB$_1$ than after *in vivo* administration of the substance (5). This may indicate that the cells in the nose, which will be engaged in AFB$_1$-bioactivation, may vary depending on the route of exposure: AFB$_1$ reaching the nose systemically via the blood may be metabolized mainly in Bowman's glands; local exposure via inhalation may result in metabolism in the cells in the surface epithelium.

The *in vitro* microautoradiography of the nose and the other tissues showed a preferential labelling of the nuclei of the cells. A similar labelling of the cellular nuclei has previously been observed in the nose of cattle and mice (5,7). It has been shown that the AFB$_1$-epoxide binds readily to double-stranded DNA (18) and the labelling of the nuclei may reflect a specific affinity of the bioactivated AFB$_1$ for the DNA.

The activation of AFB$_1$ appears to be one important determinant for the potency of its effects. Another important determinant for the susceptibility to AFB$_1$ toxicity and carcinogenicity is the activity of glutathione-S-transferase (GST) toward the AFB$_1$-8,9-epoxide. Thus, GST-activity is inversely related to the susceptibility of rodent species to AFB$_1$-hepatocarcinogenesis (19,20). In mice, extrahepatic binding of the bioactivated AFB$_1$ to tissue-macromolecules is increased when the tissue-levels of glutathione are reduced by treatments with phorone (5). Primates appear to have low levels of GST-activity in the liver (21). Studies in monkeys on the activity of GST toward the AFB$_1$-8,9-epoxide in extrahepatic tissues would be of interest.

LC analysis of lipid-soluble metabolites in the liver of the AFB$_1$-injected monkeys showed that AFM$_1$ was the dominating metabolite. In addition small amounts of AFP$_1$ were detected. AFM$_1$ was also the major lipid-soluble metabolite formed by the liver and the nasal olfactory mucosa *in vitro*. In the rhesus monkey AFM$_1$ has been shown to be the main lipid-soluble metabolite in plasma and urine (22). AFP$_1$ has also been shown to be a significant urinary AFB$_1$-metabolite in the rhesus monkey and a considerable proportion of this metabolite is present in the urine as conjugates with glucuronic acid and sulfate (23). In the rhesus monkey (24) and in *Macaca nemestrina* (25) AFQ$_1$ has been shown to be a major lipid-soluble AFB$_1$-metabolite formed by liver homogenates or subcellular liver fractions *in vitro*, whereas AFM$_1$ is formed in smaller amounts. It appears that there may be differences in the formation of lipid-soluble AFB$_1$-metabolites between monkey-species.

The autoradiography showed labelling of pigmented tissues, such as in the eyes and in hair-follicles, of the monkeys injected with ^{3}H-AFB$_1$, and LC analysis showed that the labelled material in the eye-melanin mainly was non-metabolized AFB$_1$. We have previously reported that AFB$_1$ is accumulated in the eye-melanin in pigmented mice and in trouts (26,27). Experiments with beef eye-melanin indicated that the mechanism of the binding involves hydrophobic interaction between the AFB$_1$ and the indole-nuclei of the melanin polymer (26).

The autoradiography of the monkey killed 15 min after injection of ^{3}H-AFB$_1$ showed a marked labelling of the grey matter of the brain, whereas the labelling of the white matter was low. It is probable that the radioactivity in the brain at this short interval mainly represents non-metabolized AFB$_1$. It is not known why the labelling of the cell-rich grey matter is higher than that of the lipid-rich myelin-containing white matter. However, it is apparent that the uptake in the central nervous system is not primarily dependent on accumulation of lipophilic AFB$_1$ in lipid-rich neuronal structures.

The carcinogenic potential of AFB$_1$ has not been examined in marmosets of the *Callithrix jacchus* strain. However, tumours have been induced by AFB$_1$ in other strains of monkeys. Thus, in one strain of marmosets, *Saquinus oedipomidas*, liver-tumours were observed in 3 of 16 monkeys exposed to AFB$_1$ (28). In another study, 13 monkeys of a group of 45 treated animals were reported to develop tumours (29). Liver-tumours dominated with 10 cases among rhesus monkeys. One cynomolgus monkey (*Macaca fascicularis*) developed an olfactory neuroepithelioma (29). It is possible that this tumour is related to a bioactivation of AFB$_1$ in the nasal mucosa.

The toxicological significance of the melanin-binding of AFB$_1$ is not known. However, the binding is reversible (26) and should result in slow release of AFB$_1$ into the cytoplasm of the pigmented cells and possible surrounding cells. There are, to our knowledge, no reports of melanomas in animals treated experimentally with AFB$_1$. However, most carcinogenicity studies with this compound have been performed in albino rodents, and a potential carcinogenicity towards pigmented tissues might therefore have escaped attention. It has been proposed that in addition to UV-light, xenobiotics may play a role in the induction of melanomas (30). It has been shown that the retinal pigment epithelium-choroid layer of the eye contains cytochrome P-450 and exhibits hydroxylase activity towards benzo[a]pyrene (31). Human melanocytes in culture are capable of bioactivating benzo[a]pyrene (32). Thus, AFB$_1$ may potentially be bioactivated in melanocytes. However, it might also be activated in cells adjacent to the melanocytes, which may be reached by diffusion. In the skin cytochrome P-450-enzymes have been shown immunohistochemically in several structures (33). AFB$_1$ localized in the skin can also undergo photoactivation via UV-light exposure with formation of the AFB$_1$-epoxide and DNA-adducts (34,35). There are reports that skin tumours are induced in albino mice at topical exposure to AFB$_1$ (36).

AFB$_1$ can be present in dietary components and has in addi-

tion been found in significant amounts in grain dust (37.38). Thus, exposure to AFB$_1$ may take place orally, by inhalation and via the skin. Conceivably local exposure of a tissue in which AFB$_1$ can be bio- or photoactivated can promote tumourigenesis at that site.

Acknowledgements

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